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Occurence of arbuscular mycorrhizal fungi in the roots and rhizospheric soil of *Capsicum frutescens* L. grown in the Wayanad district of Kerala, India

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ABSTRACT

The percentage of root colonization, spore count and diversity of arbuscular mycorrhizal fungi associated with the rhizospheric soil and roots of *Capsicum frutescens* growing in Wayanad region Kerala was assessed. The status of arbuscular mycorrhizal fungi was studied at five places of northern Wayanad viz, Kalpetta, Vythiri, Manathavady, Sultan Bathery, and Meenagadi of Kerala, India. Samples were collected during the month of January to February 2016. It was found that the association of AMF with roots of *Capsicum frutescens* ranged from 69-98%. Chilli plants of Vythri region showed maximum 98% colonization, followed by Sultan bathery 88%, Manathavady 79%, Kalpetta 73% and minimum colonization 69% was observed in Meenagadi. The spore density of AMF ranged from 124 to 350 spores per 100 g of soil. Percent root colonization and spore density was comparatively higher in the Vythri region than other sampling sites. A total of 17 AMF morphotypes were recorded, 9 were identified upto genus level. Among identified AMF taxa *Glomus* species were found to be dominant followed by *Acaulospora sp*. and *Gigaspora sps*. The spore density and percent colonization was highest at soil pH -7.32 in Vythiri region compared to Meenagadi study area at lowest pH- 4.55 .The soil pH and cool climate may be the contributed factors in the colonization of AMF with roots of *Capsicum frutescens.*

KEYWORDS: Arbuscular Mycorrhizal fungi, Kerala, *Capsicum frutescens*, Root colonization, Spore density.

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INTRODUCTION

The world's extent species of vascular plants are typically mycorrhizal forming the association of fundamental importance in all ecosystems.[1] The association between plant and fungi of the *Glomeromycota* is one of most widespread mutualistic symbiosis between plants and microorganisms. AMF form symbiotic association with the plant roots that assists the plant in mineral element uptake.[2] AMF form symbiotic association with the plant roots. The roots of Chilli normally form a symbiotic association with AMF.[3]AM fungi can be more beneficial for plant growth and physiology under dry conditions. The fine roots that perform most of the mineral uptake process through symbiotic association with the fungi which improve nutrient uptake and drought tolerance and to protect the plants against pathogens. The AM fungi are known to be supportive to crop plants through uptake of diffusion limited nutrients, biological control , hormone production and drought resistance.[4-5] AMF are known to colonize up to 80% of the roots of terrestial plant, thereby increasing their drought resistance as well as improving their nutrition, growth and disease tolerance.[6]

Capsicum frutescens. L (Bird's eye chilli or Chilli pepper) belonging to the family Solanaceae is a perennial herb produces small hot chillies. It is one of the important spices in Kerala locally known as Kanthari mulaku. Chilli pepper considered as one of the major source of vitamin C. The plant contains antioxidant, vitamin E and pro vitamin. It also constitutes a good source of carotenoids and xanthophylls. [7] *Capsicum frutescens* serves as an important crop of great commercial value which finds diverse use as vegetable, culinary, and medicinal purposes. Chilli pepper has two important quality parameters which gives it great commercial attraction are the red colour due to pigment Capsanthin and pungency attributed by capsaicin.[8] Association of *Glomus and Gigaspora sps* was observed in chilli peppers (*Capsicum annum. L.)* grown in Sahelian soil. *Glomus* and *Gigaspora spp* are the most common mycorrhizal species in neutral to alkaline soils.[9-10]

In the present study an attempt has been made to find the occurence of mycorrhizal association with Chilli pepper plants and identification of AM spores in the rhizospheric soil of five different regions of *Capsicum frutescens* growing area of northern Wayanad, Kerala.

MATERIALS AND METHODS:

a) Study area:

In the present field survey, five places of northern Wayanad viz, Kalpetta, Vythiri, Manathavady and Sultan Bathery, Meenagadi of Kerala state, India, were selected as study area. The root and soil samples were collected from different sites randomly .The samples were collected during the month of January - February 2016.

b) Collection of root and soil samples:

The Rhizospheric soil samples were taken from *Capsicum frutescens* plant growing area from a depth of 0-30cm from different locations in Kerala. In each study area plant along with roots and rhizospheric soil was collected in sterile polythene bags using soil auger. Approximately 500gm of rhizosphere soil was collected. Soil particles adhered to fine roots was removed by generous shaking and roots connected to each sampled plant were also collected to quantify their AM status. The root are cut into small pieces and stored in standard FAA solution to assess percent colonization.

c) Root clearing and staining:

The cleaned roots of *Capsicum frutescens* were cut into 1cm long pieces and stained with Trypan blue, according to the procedure described by .[11]The roots were autoclaved in 10% KOH for 15 min at 15 lb pressure depending on the thickness of root structure. The 10% KOH solution was poured off retaining the root bits inside the vials and the root bits were washed thrice with tap water. Later the roots were acidified with 5N HCl for 2-3 min and washed with water and stained by immersing the roots in 0.5% Trypan blue prepared in lactophenol for 10-15 min in a hot water bath, until the roots are stained satisfactorily. The root bits were mounted serially on a clean glass slide in lactophenol and observed under microscope for AMF association forming structures, arbuscules, vesicles and mycelium.

The root colonization was calculated by simple slide technique method.[12] The root pieces (1cm long) were selected at random from the stained samples and 10 root bits were mounted on glass slide and observed under compound microscope. Presence and /or absence of AM structure (Mycelium, Arbuscules and Vesicles) were recorded in each root pieces. An average of 100 root segments was used for the enumeration and assesses the percentage of root colonization using the following formula.

$$\text{Percent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

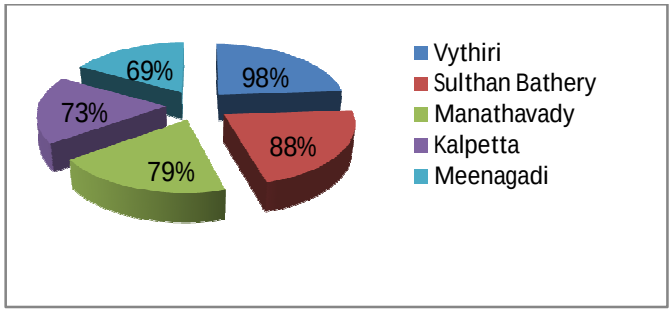
d) Isolation and identification of Arbuscular mycorrhizal Spores:

The isolation of AM fungal spores from the rhizospheric soil samples was done by wet sieving and decanting method.[13] The spores were counted under the stereo zoom microscope. Spore population was then expressed in terms of number of spores per 100 gm of dry soil. Clean and intact spores were isolated using a specially designed needle, spores were mounted with PVLG + Melzer's Reagent and observed under microscope. Taxonomic identification of spores up to species level was based on spore size, spore colour, wall layers and hyphal attachments using the species descriptions provided by INVAM [14] and other suitable references.[15-19]

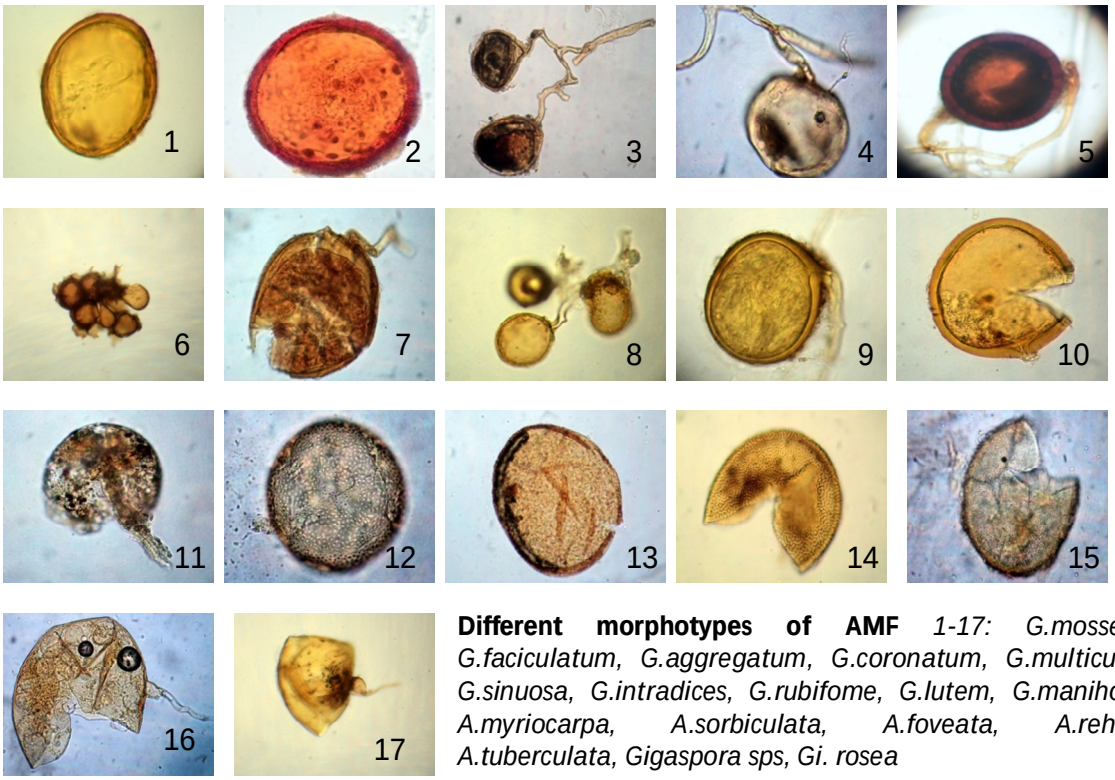
RESULTS AND DISCUSSION.

Diversity of AM Fungi associated with the rhizospheric soil and roots of *Capsicum frutescens* plants growing in wild conditions showed the percentage of AMF association ranging from 69-98% in the roots of *Capsicum frutescens* plants. In Vythri region it showed maximum 98% colonization, followed by Sulthan bathery with 88%, in Manathavady 79%, Kalpetta 73% and minimum colonization 69% was observed in Meenagadi. (Figure-1). The soil pH analysis reveals that there is a variation in the soil pH between different sampling sites. Highest soil pH 7.32 was recorded from Vythiri sample which was alkaline, where as lowest pH 4.55 was recorded from Meenagadi and was acidic in nature.

Figure 1. Percent root colonization of AM fungi with *Capsicum frutescens* collected from Northern regions of Wayanad , Kerala



Number of spore density was observed in 100 g of soil sample collected from different region in northern regionsof Kerala.It was observed that the roots *Capsicum frutescens* plants colonized by 9 AMF morphotypes with 350 spores in Vythiri region followed by Sulthan bathery showing 5 morphotypes with 323 spores. In Manathavady region the spore denisity was 269 in 5 morphotypes and Kalpetta with 158 spores in 4 morphotypes.. The least spore density was recorded in Meenagadi by 3 morphotypes with 124 spores (Table 1,Plate 1-17)



Different morphotypes of AMF *1-17: G.mosseae, G.faciculatum, G.aggregatum, G.coronatum, G.multiculae, G.sinuosa, G.intradices, G.rubifome, G.lutem, G.manihotis, A.myriocarpa, A.sorbiculata, A.foveata, A.rehmii, A.tuberculata, Gigaspora sps, Gi. rosea*

Table No 1: Number of spores and Species of AMF recorded in 100g of rhizosphere soil sample of *Capsicum frutescens* collected from Northern regions of Wayanad , Kerala

Regions	Spore No/100g soil	No.of Species	AMF Species name
Vythiri	350	9	*G.faciculatum, G.aggregatum, G.coronatum, A.myriocarpa, G.multiculae ,G.sinuosa , A.scrobiculata Gigaspora sps, Gi. rosea*
Sulthan bathery	323	5	*G.faciculatum , G.aggregatum , G.intradices , A.scrobiculata, G.clavisporum,*
Manathavady	269	6	*G.faciculatum ,A.tuberculata , A.myriocarpa, A.rehmii , G.luteum , Gi. rosea*
Kalpetta	158	4	*G.rubiforme, A.myriocarpa, G.manithotis, G.faciculatum*
Meenagadi	124	3	*G.faciculatum, G.mossae, A. foveata*

In the rhizospheric soil samples *Glomus sps* were found to be very dominant in the *Capsicum frutescens* plants. Among different morphotypes 11 *Glomus species* were found to be dominant followed by 5 species of *Acaulospora* and two species *Gigaspora*. Our experimental data indicated the occurrence of *Glomus fasciculatum* as common among all the selected regions. Variation in the spore density and percent colonization among different sampling sites depicts the specificity of host with the AMF as influenced by edaphic factors. The variation in soil pH affects

the germination of mycorrhizal spores. More number of spores was recorded in alkaline condition of soil.

Various mycorrhizal populations contribute to healthy fertile soils through the uptake of nutrients. The wide spread AM fungal associatoion could be attributed to the soil structure and climatic condition of the *Capsicum frutescens* growing area of Wayanad in Kerala. The interrelationship of plants with AM fungi colonization leads to the better survival with nutrient uptake and resistance towards the pathogens.

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